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Lymphatic-targeted cationic liposomes: A robust vaccine adjuvant for promoting long-term immunological memory

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ABSTRACT

Although retaining antigens at the injection site (the so-called "depot effect") is an important strategy for vaccine development, increasing evidence showed that lymphatic-targeted vaccine delivery with liposomes could be a promising approach for improving vaccine efficacy. However, it remains unclear whether antigen depot or lymphatic targeting would benefit long-term immunological memory, a major determinant of vaccine efficacy. In the present study, OVA antigen was encapsulated with DOTAP cationic liposomes (LP) or DOTAP-PEG-mannose liposomes (LP-Man) to generate depot or lymphatic-targeted liposome vaccines, respectively. The result of *in vivo* imaging showed that LP mostly accumulated near the injection site, whereas LP-Man not only effectively accumulated in draining lymph nodes (LNs) and the spleen, but also enhanced the uptake by resident antigen-presenting cells. Although LP vaccines with depot effect induced anti-OVA IgG more potently than LP-Man vaccines did on day 40 after priming, they failed to mount an effective B-cell memory response upon OVA re-challenge after three months. In contrast, lymphatic-targeted LP-Man vaccines elicited sustained antibody production and robust recall responses three months after priming, suggesting lymphatic targeting rather than antigen depot promoted the establishment of long-term memory responses. The enhanced long-term immunological memory by LP-Man was attributed to vigorous germinal center responses as well as increased Tfh cells and central memory $CD4^+$ T cells in the secondary lymphoid organs. Hence, lymphatic-targeted vaccine delivery with LP-Man could be an effective strategy to promote long-lasting immunological memory.

1. Introduction

Vaccination is a cost-effective approach for preventing human infectious diseases. Immunological adjuvants, as the key component of vaccines, are highly desirable for improving vaccine efficacy, especially for subunit vaccines [1,2]. An ideal vaccine adjuvant should be able to accelerate and augment vaccine-induced immune responses, modulate the type of responses (*e.g.* Th1 *versus* Th2 responses), and promote immunological memory [3]. The latter, in particular, enables the immune system to respond rapidly and vigorously upon re-exposure to the same pathogen, and provides long-lasting protection against infection [4].

Cationic liposomes are positively charged nanoparticles made from closed lipid-bilayers, and have been used as immunological adjuvants for enhancing vaccine-induced humoral and cellular immune responses [5]. The adjuvant effect of cationic liposomes is strongly associated with their capacity of retaining antigens at the injection site (the so-called "depot effect"), that allows slow and prolonged antigen exposure to immune cells in the peripheral tissue, thereby enhancing vaccine efficacy [6,7]. So far, immunological adjuvants with the depot effect, such as incomplete Freund's adjuvant and aluminum compounds, remain the major platform for vaccine development [8]. However, latest studies showed that persisting antigen depots strikingly retained effector and memory T cells at the vaccination sites, causing their dysfunction and deletion [9,10]. Hence, the effect of depot formulations on vaccine-induced immune responses, especially long-term memory, needs to be re-considered.

The spleen and lymph nodes (LNs) are secondary lymphoid organs essential for vaccine-induced immune responses. Upon

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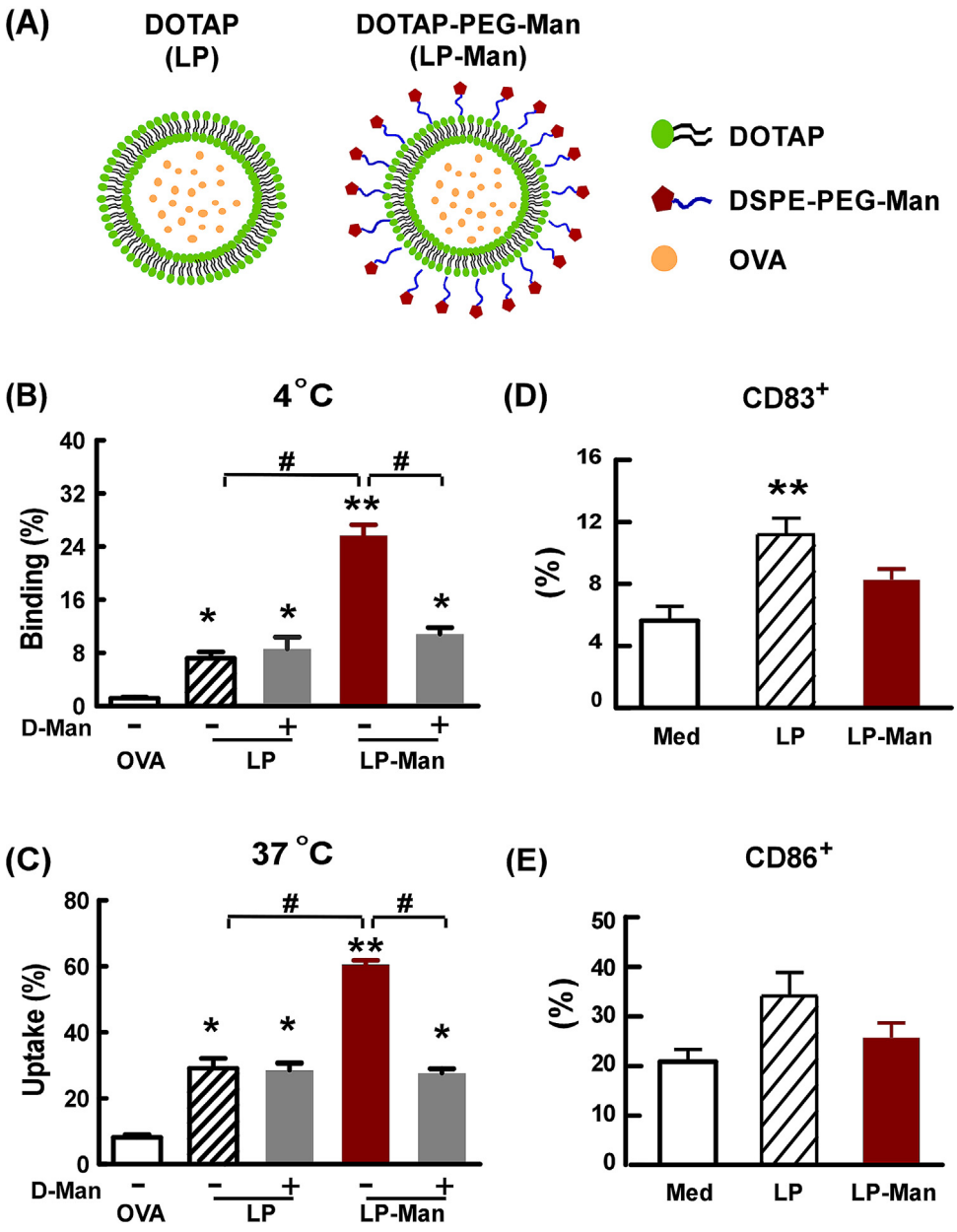

Fig. 1. The effect of different liposomes on Ag binding/uptake and maturation of mouse BMDCs. (A) Schematic of liposome vaccines. (B and C) Immature BMDCs were generated as described in Section 2, and were pre-treated with or without 0.16 mol/L of D-mannose (D-Man) solution for 1 h, followed by incubation with OVA-FITC antigen or liposomes encapsulated OVA-FITC at 4 °C or 37 °C for 30 min. The binding and uptake of OVA-FITC was measured using flow cytometry. (D and E) Immature BMDCs were cultured with X-vivo medium (Med) or 50 nmol/ml of different liposomes for 24 h, and the expressions of CD83 and CD86 were measured using flow cytometry. Bars shown are mean ± SE (n = 3–4), differences among groups were determined using One-way ANOVA analysis. The asterisks indicate differences between OVA and liposome-treated groups are statistically different. $^{*}p < 0.05$, $^{**}p < 0.01$. $^{\#}$Differences between two groups are statistically different, $p < 0.05$.

immunization, peripheral antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, capture antigens from the vaccination site, and then migrate into the secondary lymphoid organs to trigger downstream T- and B-lymphocyte activation as well as memory cell differentiation [11]. The spleen and LNs also contain a large number of resident APCs, which can actively process antigens and participate in vaccine-elicited immune responses [12,13]. Moreover, the spleen and LNs are the major reservoir for long-lived memory B cells and central memory T cells [14,15], therefore playing a crucial role in generating long-term immunological memory. Previous studies showed that intralymphatic vaccination induced IgG2a and IFN-γ more potently than subcutaneous (SC) or intramuscular (IM) injection [16]. More interestingly, intra-lymphatic immunization of *Mycobacterium bovis* BCG vaccine induced a sustained immunological protection *in vivo* [17]. These data suggest that enhancing vaccine delivery into the lymphatic system ("lymphatic targeting") might be a promising approach for improving vaccine efficacy.

Lymphatic-targeted vaccine delivery can be achieved by using g nanoparticles modified with APC-specific ligands or antibodies by PEGylation [18]. Mannose receptor (MR) is a member of calcium-dependent C-type lectin receptor (CLR) family, and primarily expressed on APCs. Previous studies showed that mannose- or oligomannose-modified nanoparticles increased vaccine delivery into draining LNs and enhanced vaccine-induced

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